

The Morphology of Bacilli at Different Levels of the Dermal Infiltrate in Lepromatous Leprosy¹

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Following Shepard and McRae's (11) demonstration that the staining character of *Mycobacterium leprae* correlates with the ability of these bacilli to multiply in mice, the Morphological Index (MI, i.e., percentage of solid and uniformly staining acid-fast bacilli) in skin smears has come to stay as an important parameter of assessment of the effect of chemotherapy, as proposed earlier by Waters and Rees (12). The MI in skin biopsy sections has been found to compare well with that of smears of the homogenates of the same tissue specimens (4). Ridley (6) mentions a higher solid staining acid-fast bacilli (AFB) count in the nerve bundles.

The present study was undertaken to see if the MI varied with the depth from the skin surface. No systematic study, to our knowledge, has so far been made on these lines.

MATERIALS AND METHODS

Skin biopsies of 20 untreated lepromatous patients were examined. In each case it was ensured that the biopsy was deep enough to include subcutaneous fatty tissue. Paraffin embedded 5 μ thick sections were stained by a modified Fite-Faraco technique (1). The MI was determined at three levels in the sections: a) the upper level of infiltrate plus the sub-epidermal free zone, b) the deepest area of granuloma, and c) in the zone approximately midway between these two levels. Using a research microscope with a $\times 100$ apochromatic objective, $\times 10$ eye pieces and critical illumination, staining characters of AFB were noted. Only those bacilli which could be seen in their entire length, lying singly, and free of background tissue staining were examined. Shepard and McRae's (11) criteria

were employed for the scoring of solid bacilli. No attempt was made to sub-classify non-solid bacilli into various groups. Two hundred bacilli were examined at each of the above levels and the MI calculated. The mean MI values at the three levels were statistically compared using the *t* test for paired values.

RESULTS

The Table shows the MI at the three levels. The mean MI at the most superficial zone (sub-epidermal area), 8.0 ± 6.5 , was found to be significantly higher than those either at the mid zone or in the deepest part of the infiltrate. The MI differences between the mid and the deep zones were not significant.

DISCUSSION

The findings reveal that there is a marked concentration of solid staining bacilli in the superficial areas and this was found to be significantly higher than the MI at the mid and deeper levels ($p < 0.001$). Though the mean MI was higher in the mid as compared to deep zone (1.33 ± 1.64 vs. 0.85 ± 1.16), the difference was not significant—thus indicating a steep fall in MI from superficial to mid zone and almost a plateau thereafter.

Similar observations of increased numbers of solid staining bacilli in the superficial levels has been made by Levy (3) and Ridley (7).

The reasons for such a non-uniform distribution of morphologically intact, i.e., solid staining bacilli, could be possibly the lower temperature at the more superficial level in the skin as compared to deeper areas. Indeed, temperature difference has been shown to affect the bacterial density in man—a clinically heavier infiltration of the cooler areas of the body is an established fact. Hastings, *et al.* (2) compared bacterial density at two nearby sites with differing temperatures in polar LL patients. They

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THE TABLE. *Morphological Index at three tissue levels.*

Section no.	Superficial part	Mid area	Deep zone
1	6.0	0	1.5
2	10.5	1.0	0
3	2.0	0	0
4	8.5	1.0	0
5	20.0	3.0	1.0
6	1.5	0	0
7	7.0	2.0	0
8	2.5	1.5	2.0
9	29.0	1.5	1.0
10	4.5	0	0
11	9.0	4.5	0
12	10.5	2.5	0
13	8.0	5.5	1.0
14	8.5	0	4.5
15	4.5	0	0
16	3.0	0	2.5
17	2.5	0	0
18	8.5	1.0	1.5
19	9.0	0	1.0
20	5.0	3.0	1.0
Mean \pm S.D.	8.0 \pm 6.5	1.33 \pm 1.64	0.85 \pm 1.16

Superficial part vs. mid area, $p < 0.001$, t test for paired samples.

Mid area vs. deep zone, $p > 0.05$, t test for paired samples.

Superficial part vs. deep zone, $p < 0.001$, t test for paired samples.

showed that the bacterial counts were significantly higher in cooler lateral sites of the back as compared to the significantly warmer mid-line at the same level. A similar explanation has been offered for relative sparing of flexural folds in lepromatous patients. In mice, multiplication of *M. leprae* has been found to be more uniform at an environmental temperature of 20°C than at a room temperature of 35°C⁽⁹⁾. The ambient temperature of 20°C resulted in foot pad temperatures of 27°C–30°C. Cool temperature probably depresses cell-mediated immunity *in vivo*. *In vitro* it has been shown by Purtilo, *et al.*⁽⁵⁾ that lymphocyte transformation is significantly suppressed (following PHA and *M. leprae* stimulation) at 33°C and 28°C when compared with blast transformation at 37°C. In addition to this, the relative vascularity of the superficial dermis may favor solid bacilli.

Another possible explanation for such a distribution could be that the most recently developed portions of lesions are those which are closest to the epidermis and this

may account for the higher MI at the superficial levels.

The above findings have practical implications—skin smears from either predominantly superficial or deeper dermal layers may not show uniform results. While doing MI in tissue sections, it will be better to include both the superficial and deeper zones to get an overall picture.

SUMMARY

The Morphological Index (MI) of bacilli at three levels of the granuloma in 20 skin biopsies from untreated lepromatous patients has been recorded. The more superficial part of the granuloma and the sub-epidermal free zone showed a significantly higher MI, compared to the mid and lower zones. Possible reasons for this non-uniform distribution of morphologically intact bacilli are discussed.

RESUMEN

Se definió el índice morfológico (IM) de los bacilos encontrados a tres niveles del granuloma en 20 biopsias de piel de pacientes lepromatosos sin tratamiento. Los bacilos de la parte más superficial del granuloma y de la zona libre subepidérmica mostraron un IM significativamente mayor que las zonas media e inferior. Se discuten las posibles razones de la distribución no uniforme de los bacilos morfológicamente íntactos.

RÉSUMÉ

On a relevé l'Index Morphologique (MI) des bacilles à trois niveaux du granulome, dans 20 biopsies cutanées obtenues chez des malades lépromateux non traités. La partie la plus superficielle du granulome, de même que la zone libre sous-épidermique, a montré un Index Morphologique significativement plus élevé, par comparaison avec les zones moyenne et plus profonde. Les raisons qui pourraient expliquer cette distribution non-uniforme des bacilles morphologiquement intacts sont discutées.

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